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Pharmacological strategies to resolve acute inflammation

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The inflammatory response is a physiological process that has the major role of restoring tissue homeostasis. However, uncontrolled or unresolved inflammation may cause tissue damage and contribute to the pathogenesis of chronic inflammatory and autoimmune diseases. Current pharmacological therapies to treat inflammatory maladies focus on inhibition of the productive phase of the inflammatory response including inhibition of leukocyte influx. Resolution of inflammation is an active process, which relies on the production of pro-resolving molecules and activation of intracellular pathways. Here, we will discuss mechanisms and therapeutic potential of pharmacological strategies, which accelerate resolution in animal models of acute inflammation by mimicking or inducing natural pathways of resolution phase of inflammation.

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Introduction

The inflammatory response is a spatially and temporally orchestrated event of innate immunity in which cells and mediators collaborate to neutralize and eliminate the damaging stimuli to allow maintenance of homeostasis [1•]. Therefore, inflammation is believed to be a physiological response, which ultimately aims to restore tissue architecture and function. In the context of infection, the initial inflammatory response may protect the host and be self-limiting, progressing to complete resolution [2]. However, if deregulated, acute inflammation may not resolve and can progress to a more chronic situation that eventually results in scarring and fibrosis. These events are believed be involved in the progression of many of the inflammatory diseases including asthma, atherosclerosis, chronic obstructive pulmonary disease and rheumatoid arthritis [3].

Resolution of inflammation is an active and continuous process, which involves activation of endogenous programs with production and activation of different biochemical mediators and signaling pathways to ensure rapid and successful restoration of tissue homeostasis [2,3,4•,5,6•] (Figure 1). In fact, resolution requires termination of the inflammatory response mainly by diminishing granulocyte recruitment; switching from pro-inflammatory mediator generation to pro-resolution mediators, including lipoxins (LXs), resolvins (Rvs), protectins, maresins, cyclopentenone prostaglandins (CyPGs), glucocorticoids (GCs), melanocortins (MCs), annexin A1 (AnxA1) and interleukin (IL)-10; turning off signaling pathways associated with cytokine production and leukocyte survival; apoptosis of recruited inflammatory cells (granulocytes) followed by nonphlogistic clearance by macrophages and reprogramming of these cells toward pro-resolution phenotypes (for review, see [6•]).

Available anti-inflammatory therapies have been developed for their capacity to inhibit or antagonize the production or action of pro-inflammatory mediators. If the early productive stages of the inflammatory response are prevented there will be prevention or slowing of the progression of inflammatory disease. For example, antibodies that target specific pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α and IL-1β, prevent local production of chemokines and expression of cell adhesion molecules, leading to decreased leukocyte influx. Importantly, certain anti-inflammatory agents such as steroids, may be considered *resolution-safe* since they promote phagocytosis of apoptotic leukocytes and increase AnxA1 release, whereas other anti-inflammatory compounds may be considered *resolution-toxic* and actually lead to longer duration of the inflammatory response [4•]. For example, cyclooxygenase (COX)-2 inhibitors block production of prostaglandin (PG)D2 and may protract resolution of inflammation [7], an event also observed after inhibition of nuclear factor (NF) kappa B [5].

A growing knowledge of the events, mediators and biochemical pathways involved in the resolution phase of inflammation has brought new opportunities to control inflammatory disease (Figure 1). Using this line of

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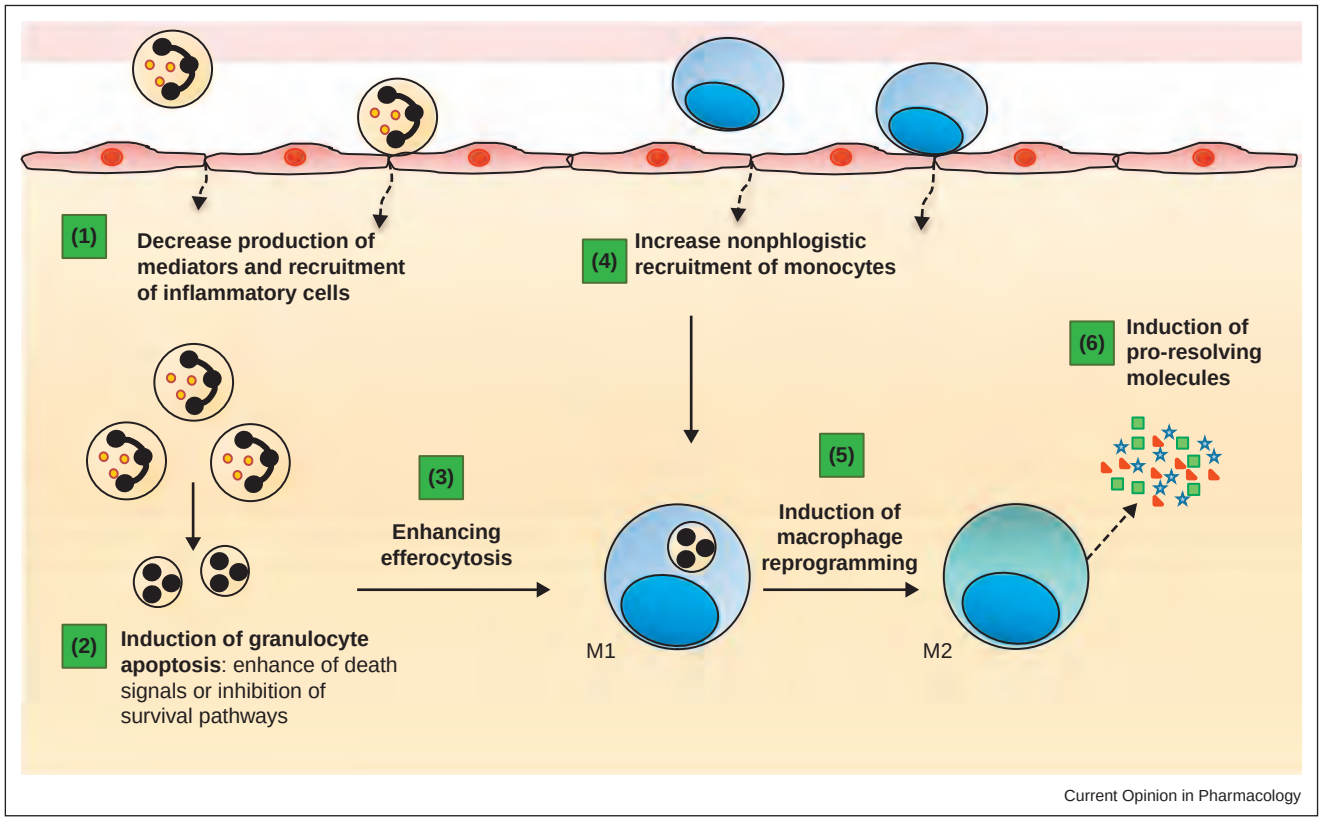
Figure 1



Turning off acute inflammation: possible pharmacological targets. There are targetable points on the resolution process (numbers on the squares). Some resolution-based therapies, for example, ATL (aspirin-triggered LX), have been proven to act in several ways to induce resolution.

thinking, there are several distinct opportunities to develop drugs that induce apoptosis, enhance efferocytosis, increase recruitment of nonphlogistic macrophage, induce reprogramming of macrophages toward a pro-resolutive phenotype capable of releasing pro-resolving molecules. As mentioned above, the concept of targeting the resolution phase of inflammation is fundamentally distinct from and may be complementary to targeting the productive phase of the inflammatory process.

Although pathways involved in apoptosis and phagocytosis of apoptotic cells *in vitro* may also be relevant in the context of an inflammatory response, it is clear that pharmacological strategies based on relevant pathways must be tested in the much more complex *in vivo* situation. Table 1 describes studies that have tested known anti-inflammatory drugs and novel compounds in the context of the resolution of inflammation. The anti-inflammatory and pro-resolving properties of endogenous and synthetic lipids derived from polyunsaturated fatty acids have been extensively investigated and reviewed by Prof. Serhan's group and others, and will not be highlighted herein. These specialized lipid mediators limit neutrophilic accumulation and enhance influx and function of pro-resolutive macrophages. As such, pro-resolving lipids have major anti-inflammatory effects in various animal models of acute and chronic inflammation [8•,9,10,11•,12–15]. Studies have reported that 15-deoxy-Delta12,14-prostaglandin J2 (15d-PGJ2), a COX2-derived CyPG, has anti-inflammatory and potentially pro-resolutive actions [16,17]. CyPGs accelerate resolution of the inflammation by preventing cell recruitment and inducing apoptosis via both peroxisome proliferator-activated receptor gamma (PPARγ)-dependent and independent mechanisms. MC peptides and their receptors (MC_1 and MC_3) have also been appreciated as anti-inflammatory in many experimental models of acute and chronic inflammation [18]. In fact, α-melanocyte-stimulating hormone (MSH) analog AP214 has been shown to resolve zymosan-induced peritonitis, reduce clinical score in experimental arthritis and induce efferocytosis *in vivo* [19]. Given the importance of endogenous mechanisms of resolution acting as *stop signal* of the inflammatory response, many pharmacological strategies are based on the concept that agonists or inducers of pathways/molecules of the resolving phase of inflammation will promote or potentiate resolution.

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Table 1

Summary of proresolving strategies trialed in animal models of acute inflammation

Administration of drugs/compounds	Effects on resolution of inflammation	Animal model of inflammation	References
Glucocorticoids	Decrease leukocyte accumulation, induce granulocyte apoptosis	Pleurisy	[38••,23]
Rolipram and cAMP elevating agents	Decrease leukocyte accumulation and induce granulocyte apoptosis	Pleurisy	[23,24]
LXs and ATLs (aspirin-triggered LX)	Decrease leukocyte accumulation, induce neutrophil apoptosis and efferocytosis, enhance bacterial phagocytosis, increase IL10 production	Peritonitis, intestinal ischemia/reperfusion, ALI (acute lung injury)	[8•,9,10]
Resolvins (RvE1 and RvD1)	Decrease neutrophil infiltration, increase neutrophil apoptosis and efferocytosis, mitigation of pro-survival signals, potentiates antibiotic clearance of bacteria	Allergic airway inflammation, ALI, inflammatory pain	[11•,12–14,29••]
Protectins	Decrease neutrophil infiltration, increase phagocytosis of apoptotic neutrophil and zymosan particles	Peritonitis	[14,29••]
Maresins (by using MaR1 a synthetic compound)	Decrease neutrophil infiltration, enhance uptake of apoptotic neutrophils by human macrophages and reduce neuropathic pain	Peritonitis	[15]
AnxA1, its bioactive peptide Ac2-26 or superAnxA1	Decrease leukocyte accumulation, induce neutrophil apoptosis and efferocytosis, increase IL-10 production	Pleurisy, peritonitis, arthritis, intestinal ischemia/reperfusion	[9,34,35,37••,38••]
Pro-oxidants (H_2O_2)	Decrease leukocyte accumulation and induce neutrophil apoptosis	Adjuvant-induced arthritis	[21]
PGD2 and 15d-PGJ_2	Decrease leukocyte accumulation and induce leukocyte apoptosis	Pleurisy, peritonitis	[16,17]
CDK inhibitors (R-roscovitine, AT7519)	Decrease leukocyte accumulation and induce granulocyte apoptosis	Pleurisy, arthritis, bleomycin-induced lung injury, meningitis	[25,26••,28]
HDAC inhibitors (valproic acid and sodium butyrate)	Decrease leukocyte accumulation and induce granulocyte apoptosis AnxA1-dependently	Peritonitis	[36]
PI3K inhibitors (LY294002, Wortmannin)	Decrease granulocyte accumulation and induce neutrophil and eosinophil apoptosis	Pleurisy	[22–24]
NF-κB inhibitors (gliotoxin, SN-50)	Decrease granulocyte accumulation and induce granulocyte apoptosis	Adjuvant-induced arthritis, pleurisy	[21,23]
ERK1/2 inhibitor (PD98059)	Decreases leukocyte accumulation and induces granulocyte apoptosis	Pleurisy	[20]
α-MSH (by using the agonist AP214)	Inhibits neutrophil infiltration and increases efferocytosis of human apoptotic neutrophil	Peritonitis, arthritis	[19]

Inhibitors of intracellular survival pathways

We and others have shown that apoptosis precedes and plays a major role in the resolution of acute neutrophilic and eosinophilic inflammation *in vivo* [2,20–25,26••]. Apoptotic cells dampen inflammatory signals by sequestering chemokines, releasing molecules that inhibit further granulocyte influx and attract monocytes, and by reprogramming macrophages [2,27•]. Several molecular pathways regulate leukocyte survival and death during inflammatory responses. However, definite evidence for the participation of intracellular signaling pathways for the resolution of inflammation in animal models exists only for a few pathways (Table 2). Understanding mechanisms that regulate apoptosis *in vivo* is vital in providing clues at which molecular pathways one should focus to develop novel therapeutic strategies. For instance, inhibitors of phosphoinositide-3 kinase (PI3K), extracellular-signal-regulated kinase (ERK1/2) and NF-κB administered at the peak of leukocyte influx promote resolution in acute models of inflammation by enhancing apoptosis of inflammatory cells [20–24]. In addition, the group of Prof. Rossi has explored the therapeutical potential of cyclin-dependent-kinase inhibitors (CDKi) (R-roscovitine and AT7519) in animal models of neutrophil and eosinophil-mediated inflammation and shown that these agents promote caspase-dependent resolution of inflammation [25,26••]. Moreover, R-roscovitine administered concomitantly with antibiotic therapy improved the resolution of the inflammation driven by pneumococcal infection and accelerated recovery [28], suggesting that apoptosis based therapy may be useful along with the conventional approach, thus increasing efficacy of treatment of infection-associated inflammatory disease. This great potential of pro-resolving-based therapy in infectious diseases has been reinforced by another study with resolvin D1 [29••].

Glucocorticoids and AnxA1 modifying compounds

GCs are the most important drugs that have been developed therapeutically for the treatment of many of the inflammatory conditions and represents the first

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Table 2

Signaling pathways shown to be relevant for resolution of inflammation *in vivo*

Signaling pathways	Effects on granulocytes	References
NF-κB	Anti-apoptotic	[21,23]
PI3K/Akt	Anti-apoptotic	[22–24]
ERK1/2	Anti-apoptotic	[20]
CDKs	Anti-apoptotic	[25,26••,28]
cAMP/PKA	Pro-apoptotic	[23,24]

successful exploitation of an endogenous anti-inflammatory mediator, cortisol. The actions of endogenous GC are complex and depend on the induction of anti-inflammatory regulatory proteins as well as inhibition of signaling pathways such as NF-κB and AP-1 [30••,31,32]. Among the GC-induced proteins, AnxA1 has been shown to have anti-inflammatory and pro-resolving properties in various animal models of inflammation and in physiological conditions [9,33–36,37••,38••]. In fact, AnxA1 limits initial steps of inflammation, specifically recruitment of leukocytes and generation of mediators [30••]. AnxA1 also acts on the resolution phase of inflammation by inducing apoptosis of neutrophils and increasing efferocytosis by macrophages. Both events were first reported *in vitro* [39,40] and validated in *in vivo* settings [37••,38••]. Importantly, the production and action of AnxA1 are involved in pro-resolutive effects of GCs [33,38••] and histone deacetylase (HDAC) inhibitors [36]. In fact, administration of HDAC inhibitors such as valproic acid and sodium butyrate at the peak of the zymosan-induced peritonitis accelerated resolution in wild type, but much more modestly in AnxA1 null mice. These effects of HDAC inhibitors are through apoptosis and efferocytosis mediated by AnxA1 [36]. Our group [38••] and others have explored the therapeutical potential of Ac2-26 peptide, an AnxA1 peptidomimetic that retains the biological activity of the whole protein. The administration of Ac2-26 during established lipopolysaccharide (LPS)-induced pleurisy promoted caspase-dependent resolution of neutrophilic inflammation associated with the induction of neutrophil apoptosis. Mechanistically, Ac2-26 resolved inflammation shifting the balance of LPS-elicited pro-survival signals toward apoptosis of inflammatory cells. Moreover, AnxA1 drove natural and dexamethasone-induced resolution of inflammation [38••].

Cleavage of AnxA1 inactivates the protein and is associated with loss of pro-resolving activities. The injection of anti-inflammatory drugs such as dexamethasone and the phosphodiesterase $(PDE)_4$ inhibitor, rolipram, prevented AnxA1 cleavage and this was associated with resolution of neutrophilic inflammation [38••]. In fact, AnxA1 cleavage resistant protein was more effective in ameliorating several aspects of inflammation [34,35], reinforcing the idea that either AnxA1 cleavage resistant mutant, its peptidomimetics or drugs that induce anxA1 or prevent its cleavage *in vivo* may represent a powerful anti-inflammatory and pro-resolving strategy for the treatment of inflammatory diseases, retaining at least in part, some of the anti-inflammatory and pro-resolutive properties of GCs. In addition to AnxA1, there are other GC-induced proteins with anti-inflammatory properties including GC-induced leucine zipper (GILZ) and mitogen-activated protein kinase phosphatase (MKP-1) whose pro-resolving abilities remain to be unveiled [30••,31,32].

Phosphodiesterase 4 inhibitors and other cAMP elevating agents

Cyclic adenosine monophosphate (cAMP) has been shown to play an important role in the immune system, usually promoting suppressive effects on the functions of inflammatory cells [41]. During recent years, there is a growing body of evidence showing that cAMP is also involved in resolution of inflammation [23,24,42•,43]. Studies have reported that cAMP induces a switch of pro-inflammatory macrophages into resolution-phase macrophages [42•] and that lower levels of cAMP and adenosine may account for the phenotype observed in a murine model of nonresolving inflammation [43]. In addition, we have described in murine models of neutrophil and eosinophil-mediated inflammation that cAMP elevation promoted by administration of rolipram or by cAMP mimetic drugs induced resolution of both eosinophilic and neutrophilic inflammation. Rolipram-mediated resolution was PKA-dependent and due to caspase-dependent granulocyte apoptosis and associated with inhibition of the PI3K/Akt and modulation of apoptosis-controlling proteins [23,24]. cAMP also seems to be part of pro-resolving abilities of MC peptides [19] and lysophosphatidylserine (lyso-PS) [44]. Recently, we have also described that rolipram-induced resolution of neutrophilic inflammation was related to increased accumulation of AnxA1 [38••]. Thus, the pro-resolving role of cAMP modulating agents found in our *in vivo* models is in line with other studies and reinforces the idea that cAMP elevating drugs may be a useful therapeutic strategy to induce resolution of inflammation.

Reactive oxygen species (ROS) and resolution

We have recently shown by both genetic and pharmacological approaches a role for hydrogen peroxide (H_2O_2) in resolving neutrophilic inflammation in a murine model of antigen-induced arthritis [21]. In this model, the peak of neutrophil influx occurs at the same time (24 hours) as that of H_2O_2 production. More importantly, there was delayed resolution in *gp91phox−/−* mice or after administration of catalase to wild-type animal (strategies which impair the production of ROS) suggesting that H_2O_2 contributes to natural neutrophil clearance. Animals that were treated with either low dose H_2O_2 or superoxide dismutase (SOD) at the peak of the inflammatory process (thereby increasing the local levels of H_2O_2) had

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enhanced resolution of inflammation concurrent with an increased number of apoptotic neutrophils and accumulation of the pro-apoptotic protein Bax and activated caspase-3. Inhibition of Akt phosphorylation and decreased NF-κB p65 translocation to the nucleus appeared to be the major mechanisms by which H_2O_2 affected neutrophil survival in murine antigen-induced arthritis [21]. Administration of intravenous immunoglobulin preparations, which are beneficial therapeutic agents in the treatment of autoimmune systemic inflammatory diseases, may also increase the production of intracellular H_2O_2 and induce apoptosis of LPS-stimulated neutrophils *in vitro* [45]. As such pharmacological modulation of H_2O_2 production may represent a novel therapeutic target to modulate neutrophilic inflammation.

Efferocytosis management

Macrophages are thought to be the mastermind cells that orchestrate the series of events leading to successful resolution of inflammation. During the onset of inflammation macrophages exhibit pro-inflammatory responses to pathogens and tissue injury, whereas during the resolution phase they promote clearance of apoptotic granulocytes, produce anti-inflammatory cytokines, pro-resolving mediators and limits dysregulated tissue repair/fibrosis [27•,44]. Nonphlogistic phagocytosis of apoptotic cells by macrophages (efferocytosis) is a critical process in the resolution program. Disintegration of late apoptotic cells releases toxic intracellular contents that protract inflammation and defective apoptotic cell clearance is associated with many autoimmune and chronic inflammatory disorders [2,3]. A body of *in vitro* and *in vivo* evidence shows that phagocytes that consume apoptotic granulocytes switch toward a pro-resolutive phenotype that suppresses the inflammatory response.

It has been proposed recently that enhancing efferocytosis may be a therapeutic strategy to induce resolution of inflammation [44,46••]. In fact, several pro-resolutive mediators such as short lived lipids, autacoids, AnxA1 and α-MSH peptides, have been shown to increase nonphlogistic phagocytosis of PMN by macrophages *in vivo* supporting a pro-resolutive role for efferocytosis in inflammation [8•,36,37••]. Frasch and Bratton [44] reviewed the role of lyso-PS in accelerating resolution of inflammation by increasing efferocytosis of apoptotic neutrophil by macrophage. Of note, the original articles described that neutrophils from patients with chronic granulomatous disease (CGD) had ineffective generation of lyso-PS associated to low levels of efferocytosis resulting in impaired apoptotic cell removal and prolonged neutrophilia [47,48]. Moreover, Schutters *et al.* [46••] presented an strategy to increase 'eat me' signals on the surface of apoptotic cells by targeting cell surface-expressed phosphatidylserine (PS) with a variant of annexin A5 (RGD-AnxA5) that enhanced efferocytosis by macrophages and increase IL-10 *in vivo*. Thus, efferocytosis management provides a tightly regulated signal for clearance of leukocyte in acute and chronic inflammation and may be useful for drug development strategies.

Conclusions and perspectives

Resolution of inflammation is an active process, which involves key events including leukocyte apoptosis, recognition and efferocytosis, and switch of macrophage phenotype from pro-inflammatory toward to a pro-resolution profile. There are now several proof-of-concept *in vivo* studies clearly showing that resolution-based strategies are effective in preclinical models of human disease. Further studies are clearly needed to identify most effective inducers of resolution, define molecular pathways involved in the process and whether these are amenable to drug development, and ultimately translate current preclinical studies into human disease. In this regard, a limitation in the area has been the use of animal models of inflammation, which are in general self-limiting. In fact, most *in vivo* studies evaluate mediators and pathways associated with resolution using strategies to accelerate or block the recovery in systems, which naturally tend to resolve. However, the studies in acute models of self-resolving inflammation have allowed mechanistic studies and provided crucial clues, which should be exploited further in more chronic nonresolving models of inflammation. We also need to know how much the enhancement of resolution will impart on fibrogenesis and whether restoration of tissue function will indeed occur if resolution is switched on earlier with pro-resolutive strategies. Such knowledge in conjunction with the availability of orally available pro-resolving molecules should certainly facilitate translation and ultimately develop a new class of anti-inflammatory drugs.

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